Topical Review

Molecular system bioenergetics: regulation of substrate supply in response to heart energy demands

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This review re-evaluates regulatory aspects of substrate supply in heart. In aerobic heart, the preferred substrates are always free fatty acids, and workload-induced increase in their oxidation is observed at unchanged global levels of ATP, phosphocreatine and AMP. Here, we evaluate the mechanisms of regulation of substrate supply for mitochondrial respiration in muscle cells, and show that a system approach is useful also for revealing mechanisms of feedback signalling within the network of substrate oxidation and particularly for explaining the role of malonyl-CoA in regulation of fatty acid oxidation in cardiac muscle. This approach shows that a key regulator of fatty acid oxidation is the energy demand. Alterations in malonyl-CoA would not be the reason for, but rather the consequence of, the increased fatty acid oxidation at elevated workloads, when the level of acetyl-CoA decreases due to shifts in the kinetics of the Krebs cycle. This would make malonyl-CoA a feedback regulator that allows acyl-CoA entry into mitochondrial matrix space only when it is needed. Regulation of malonyl-CoA levels by AMPK does not seem to work as a master on–off switch, but rather as a modulator of fatty acid import.

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In muscle cells contractile function and cellular energetics are fuelled by oxidation of carbohydrates and fatty acids (Bing, 1965; Neely *et al.* 1972; Neely & Morgan, 1974; Williamson *et al.* 1976; Williamson, 1979; Opie, 1998; Rasmussen & Wolfe, 1999; Stanley *et al.* 2005; Taegtmeyer *et al.* 2005; Kiens, 2006). The choice of substrates depends upon their availability (Neely & Morgan, 1974; Williamson, 1979; Opie, 1998; Rasmussen & Wolfe, 1999; Roden, 2004; Stanley *et al.* 2005; Taegtmeyer *et al.* 2005; Kiens, 2006), and the rates of their utilization are very precisely regulated by multiple interactions between the intracellular compartmentalized and integrated bioenergetic systems of glycolysis, fatty acid oxidation and the Krebs cycle in the mitochondrial matrix, linked directly to the activity of the respiratory chain and the phosphorylation process catalysed by the ATP synthase complex (Neely & Morgan, 1974; Williamson, 1979; Randle, 1998; Stanley *et al.* 2005; Taegtmeyer *et al.* 2005; Kiens, 2006). The rates of all these processes are geared to the workload, mostly by the mechanism of feedback metabolic regulation (Neely & Morgan, 1974; Williamson, 1979; Randle, 1998). These complex systems and the mechanisms of their regulation are the ideal subjects of studies for molecular system bioenergetics, which deals with the quantitative description of integrated and organized cellular systems, taking into account the multiple interactions between different parts of the whole system (Noble, 2002; Kitano, 2002; Saks *et al.* 2006).

In a previous recent review (Saks *et al.* 2006), we showed that the molecular system approach to the study of cellular bioenergetics has been very useful in elucidating the mechanism of feedback regulation of mitochondrial respiration in cardiac cells, which is the metabolic basis of the Frank-Starling law of the heart and explains the workload-dependent increase of respiration under conditions of metabolic stability. Here, we evaluate the mechanisms of regulation of substrate supply for mitochondrial respiration in muscle cells, and show that a system approach is useful also for revealing mechanisms of feedback signalling within the network of substrate oxidation and for explaining the role of malonyl-CoA in regulation of fatty acid oxidation in cardiac muscle. Analysis of the results of multiple experimental studies in their historical perspective, from the point of view of molecular system bioenergetics, shows that malonyl-CoA has most probably the main role in feedback metabolic

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inhibition of fatty acid oxidation, similar to many other metabolic feedback inhibitors or activators, but does not seem to be the key regulator of fatty acid oxidation *per se*, as suggested in many recent works (Dyck & Lopaschuk, 2002, 2006; Cuthbert & Dyck, 2005; Wolfgang *et al.* 2006; Hardie & Sakamoto, 2006). The principal key regulator of energy metabolism, including fatty acid oxidation in normal muscle cells, is the energy demand or the workload, which controls the mitochondrial oxidative phosphorylation processes by a mechanism of metabolic feedback regulation. For this, multiple metabolic intermediates are involved in setting the steady state rates of substrate supply (Neely *et al.* 1972; Neely & Morgan, 1974; Williamson *et al.* 1976; Williamson, 1979; Randle, 1998; Stanley *et al.* 2005; Saks *et al.* 2006).

Short historical perspective

Probably, Clark *et al.* (1931) were the first to show, on isolated frog heart, that the oxidation of carbohydrates explains not more than 40% of oxygen uptake. In 1954, Bing and his coworkers demonstrated, by using coronary sinus catheterization, the absolute requirement of the human heart for free fatty acids (HFFA) as fuel (Bing *et al.* 1954; Bing, 1965). These investigations opened the field for intensive studies on cardiac metabolism and led to the development of molecular and cellular cardiology. The mechanisms of regulation of the substrate uptake were intensively studied by Opie *et al.*, Neely, Morgan, Williamson, Randle and very many others (Neely *et al.* 1972; Oram *et al.* 1973; Neely & Morgan, 1974; Williamson *et al.* 1976; Williamson, 1979; Randle, 1998; Opie, 1998; Rasmussen & Wolfe, 1999; Beauloye *et al.* 2002; Roden, 2004; Reszko *et al.* 2004; Stanley *et al.* 2005; Taegtmeyer *et al.* 2005; King *et al.* 2005; Kiens, 2006). In general, the results of all these studies show that in heart, in the presence of both carbohydrate substrates and FFAs, about 60–90% of the oxygen consumed is used for oxidation of free fatty acids, and that the rates of both oxygen consumption and fatty acid oxidation increase linearly with the elevation of the workload (Bing, 1965; Neely *et al.* 1972; Oram *et al.* 1973; Neely & Morgan, 1974; Williamson *et al.* 1976; Williamson, 1979; Opie, 1998; Stanley *et al.* 2005). In cardiac muscle all these important changes occur at unchanged global levels of ATP, phosphocreatine (PCr), ADP and AMP (Neely *et al.* 1972; Oram *et al.* 1973; Williamson *et al.* 1976; Beauloye *et al.* 2002). The main mechanisms of the regulation of fatty acid and glucose oxidations were discovered and described in fundamental works of the groups of Neely, Randle, Williamson and others, and have been described in detail in a series of reviews (Neely *et al.* 1972; Williamson *et al.* 1976; Williamson, 1979; Randle, 1998).

On the other hand, it was discovered some 20 years ago that one of the intermediates of fatty acid metabolism, malonyl-CoA, is an effective inhibitor of the carnitine palmitoyltransferase I (CPT I), and thus of the transfer of acyl groups into mitochondria for β-oxidation (for reviews see Stanley *et al.* 2005; Cuthbert & Dyck, 2005; Hardie & Sakamoto, 2006). Malonyl-CoA is produced from acetyl-CoA through the acetyl-CoA carboxylase (ACC) reaction, and in the malonyl-CoA decarboxylase (MCD) reaction converted back into acetyl-CoA (Lysiak *et al.* 1988; Saddik *et al.* 1993; DiLisa *et al.* 1995; Schonekess & Lopaschuk, 1995; Reszko *et al.* 2004; Cuthbert & Dyck, 2005; King *et al.* 2005). More recently, it became clear that ACC is inhibited upon its phosphorylation by AMP-activated protein kinase AMPK (Winder & Hardie, 1996; Winder *et al.* 1997; Dyck & Lopaschuk, 2002, 2006; Stanley *et al.* 2005; Hardie & Sakamoto, 2006). This reaction sequence served as the basis for a hypothesis that was advocated in numerous recent works, according to which malonyl-CoA is a key regulator of fatty acid oxidation (Saddik *et al.* 1993; Rasmussen & Wolfe, 1999; Reszko *et al.* 2004; Cuthbert & Dyck, 2005; Wolfgang *et al.* 2006; Hardie & Sakamoto, 2006; Kiens, 2006; Dyck & Lopaschuk, 2002, 2006).

Paradoxically, this popular hypothesis contradicts the classical results of the previous decades discussed above. It logically follows from this hypothesis that under normal aerobic conditions (high PCr and very low AMP intracellular concentrations) in the heart, fatty acid oxidation should be inhibited and carbohydrates should be the main substrates for oxidation. This would be contrary to all classical work and observations, which have consistently shown that under normoxic conditions in the heart, FFA oxidation is strongly increased with the elevation of workload at stable levels of ATP, PCr and AMP (Neely *et al.* 1972; Oram *et al.* 1973). An increasing amount of evidence supports this conclusion and shows the need to solve this contradiction. Already in 1999, Goodwin and Taegtmeyer did not observe any changes in ACC activity during workload changes induced by adrenaline and elevation of afterload, but nevertheless observed an increase in fatty acid oxidation and a decrease in the malonyl-CoA content in the isolated working rat heart preparation (Goodwin & Taegtmeyer, 1999). More recently, King *et al.* (2005) reported similar results: an elevation of the workload increased fatty acid oxidation and in parallel resulted in a decrease of malonyl-CoA, but with no changes observed in the actual activities of ACC and AMPK. Very similar data were obtained in the laboratory of Hue, when glucose was used as a substrate (Beauloye *et al.* 2002). The authors used an isolated working heart preparation and observed perfect metabolic stability during abrupt increase in the workload: no changes in the PCr content and even a decrease in the AMP/ATP ratio (Beauloye *et al.* 2002) that excluded

any participation of AMPK in the regulation of heart energetics under these conditions. Thus, the unsolved question, the real problem is: how can β-oxidation proceed in the aerobic heart and supply the metabolic energy needed for cardiac function, in spite of inhibition of the entry of the necessary acyl groups into mitochondria by malonyl-CoA?

Solution of the problem: kinetic changes in the Krebs cycle during workload elevation

There are useful and significant results in earlier work which are sometimes forgotten but which can help to solve questions that are actively discussed these days. For example, Fig. 1 shows such data taken from the works of Neely *et al.* published in 1972 and 1973 (Neely *et al.* 1972; Oram *et al.* 1973). The experiments described were performed on isolated Langendorff-perfused rat hearts at two workloads and correspondingly at two oxygen consumption rates. The hearts were perfused, with the perfusate containing both glucose (11 mM) and palmitate (1.2–1.4 mM). Under these conditions, as was directly shown by the authors, no changes were observed in the content of ATP, phosphocreatine and creatine, which always stayed unchanged during the experiment, and the AMP content was very low and not changed

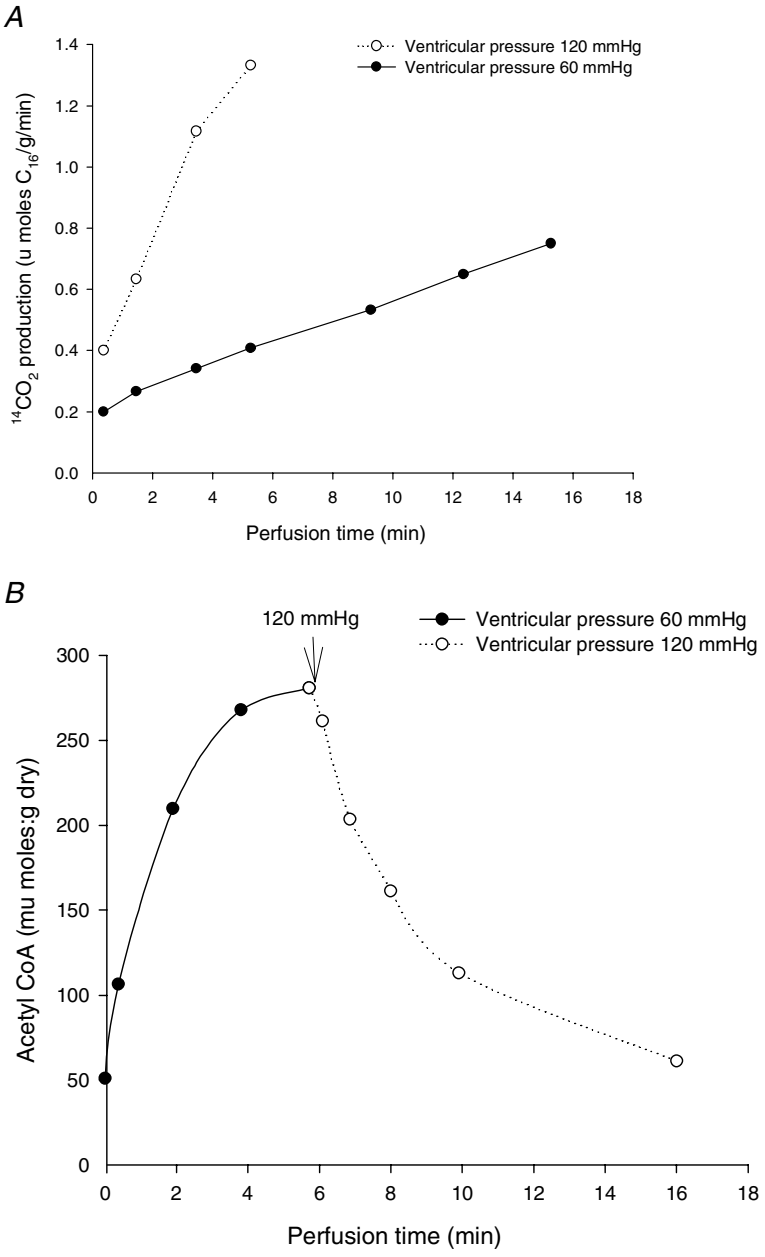

Figure 1. Workload-dependent increase of fatty acid oxidation and simultaneous decrease of acetyl-CoA in cardiac
A, increase of the $^{14}CO_2$ production from [U-^{14}C]palmitate in Langendorff-perfused isolated rat hearts with elevation of aortic perfusion pressure from 60 mmHg (continuous line) to 120 mmHg (dotted line). Hearts were perfused with a buffer containing 11 mM glucose, albumin (3%) and 1.4 mM [U-^{14}C]palmitate. The oxygen consumption rate increased from 30 to 70 μmol min^{-1} gdw^{-1} after ventricular pressure development was increased as shown above. Data are taken from Oram *et al.* (1973). *B*, effect of ventricular pressure development on the tissue levels of acetyl-CoA in Langendorff-perfused isolated rat hearts. Hearts were perfused with buffer containing 11 mM glucose, albumin (3%) and 1.2 mM palmitate. After 6 min of perfusion with aortic perfusion pressure 60 mmHg (continuous line) the pressure was elevated to 120 mmHg (dotted line). Data are taken from Oram *et al.* (1973).

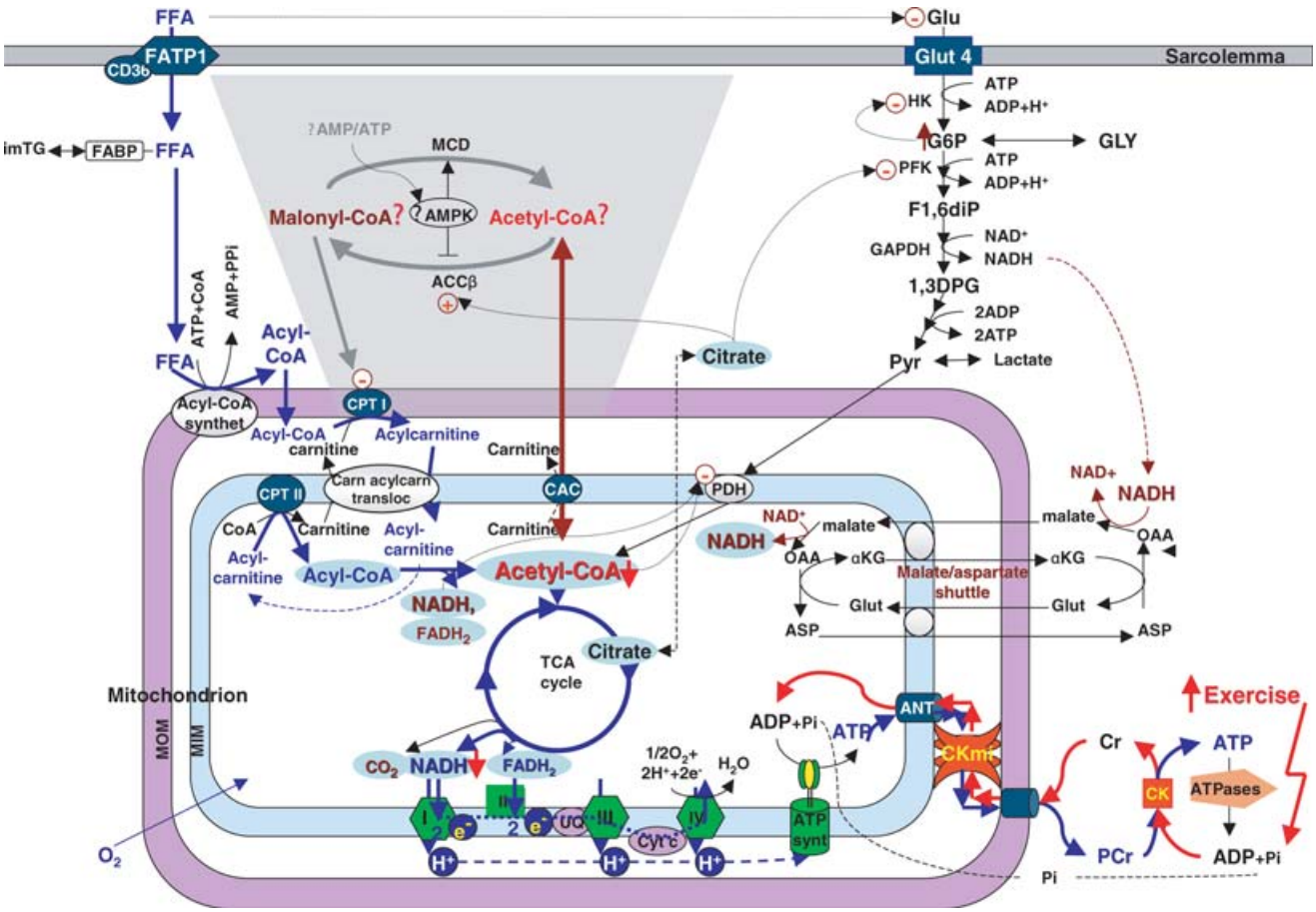

Figure 2. The scheme of substrate supply for mitochondrial respiration and the mechanisms of feedback regulation of fatty acid and glucose oxidation during workload elevation in oxidative muscle cells: central role of TCA cycle intermediates

FFAs are taken up by a family of plasma membrane proteins (fatty acid transporter protein (FATP1), fatty acid translocase (CD36)) and in cytoplasm associated with fatty acid binding protein (FABP). FFAs are esterified to acyl-CoA via fatty acyl-CoA synthetase. The resulting acyl-CoA is then transported through the inner membrane of the mitochondrion, via the exchange of CoA for carnitine by carnitine palmitolyltransferase I (CPT I). Acylcarnitine is then transported by carnitine acylcarnitine translocase into the mitochondrial matrix where a reverse exchange takes place through the action of carnitine palmitoyltransferae II (CPT II). Once inside the mitochondrion acyl-CoA is a substrate for the β-oxidation pathway, resulting in acetyl-CoA production. Each round of β-oxidation produces 1 molecule of NADH, 1 molecule of $FADH_2$ and 1 molecule of acetyl-CoA. Acetyl-CoA enters the TCA cycle, where it is further oxidized to CO_2 with the concomitant generation of 3 molecules of NADH, 1 molecule of $FADH_2$ and 1 molecule of ATP. Acetyl-CoA, which is formed in the mitochondrial matrix, can be transferred into the cytoplasm with participation of carnitine, carnitine acetyltransferases and carnitine acetyltranslocase (carnitine acetylcarnitine carrier complex, CAC). Glucose (GLU) is taken up by glucose transporter-4 (GLUT-4) and enters the Embden-Meyerhof pathway, which converts glucose via a series of reactions into 2 molecules of pyruvate (PYR). As a result of these reactions, a small amount of ATP and NADH are produced. G6P – glucose 6-phosphate; HK – hexokinase; PFK – phosphofructokinase; GLY – glycogen; F1,6diP – fructose-1,6-bisphosphate; GAPDH – glyceralhehydephosphate dehydrogenase; 1,3DPG – 1,3 diphosphoglycerate. The redox potential of NADH is transferred into the mitochondrial matrix via the malate–aspartate shuttle. OAA – oxaloacetate; Glut – glutamate; αKG – α-ketoglutarate; ASP – aspartate. Malate generated in the cytosol enters the matrix in exchange for α-ketoglutarate (αKG) and can be used to produce matrix NADH. Matrix oxaloacetate (OAA) is returned to the cytosol by conversion to ASP and exchange with glutamate (Glut). Most of the metabolic energy derived from glucose can come from the entry of pyruvate into the Krebs cycle and oxidative phosphorylation via acetyl-CoA production. NADH and $FADH_2$ are oxidized in the respiratory chain (complexes I, II, III and IV). These pathways occur under aerobic conditions. Under anaerobic conditions, pyruvate can be converted to lactate. Feedback regulation of substrate supply occurs in the following way. A glucose–fatty acid cycle (Randle hypothesis): if glucose and FFAs are both present, FFAs inhibit the transport of glucose across the plasma membrane, acyl-CoA oxidation increases the mitochondrial ratios of acetyl-CoA/CoA and of NADH/NAD^+, which inhibit the pyruvate dehydrogenase (PDH) complex, and increased citrate (produced in the Krebs cycle) can inhibit phosphofructokinase (PFK). These changes

at all (Neely *et al.* 1972; Oram *et al.* 1973). This phenomenon – metabolic stability (homeostasis) of the heart at different workloads – was confirmed later by numerous investigations and is one of the most important characteristics of cardiac muscle metabolism (Balaban *et al.* 1986; Balaban & Heineman, 1989; Beauloye *et al.* 2002; Saks *et al.* 2006). Figure 1*A* shows that under these conditions of metabolic stability, the increase in workload is paralleled by a linearly increased uptake and metabolism of palmitate. Analysis of the changes in the intermediates of fatty acid oxidation showed that the workload-dependent increase of palmitate uptake and oxidation is accompanied by a very significant decrease of the acetyl-coenzyme A content (Fig. 1*B*). This reflects the kinetics of the reactions in the Krebs cycle in response to an increase in the rate of respiration and NADH oxidation relative to acyl-carnitine transport into mitochondria and β-oxidation (Neely & Morgan, 1974). These results may explain the real role of the malonyl-CoA in heart. They show that among the metabolic changes in the heart at high workloads, one of the most remarkable is a decrease of the content of acetyl-CoA, in consequence leaving ACC with much less substrate and thus resulting in a significant decrease of malonyl-CoA in the presence of active MCD. Acetyl-CoA, which is formed in the mitochondrial matrix space, is transferred into the cytoplasm with participation of carnitine, carnitine acetyltransferases and carnitine acetyltranslocase (carnitine acetyl carnitine carrier, CAC in Fig. 2) to the cytoplasm. These reactions have been described by many authors, and they seem to be in rapid equilibrium (Marquis & Fritz, 1965; Fritz & Marquis, 1965; Neely & Morgan, 1974; Lysiak *et al.* 1988; Saddik *et al.* 1993; DiLisa *et al.* 1995; Schonekess & Lopaschuk, 1995; Poirier *et al.* 2002; Reszko *et al.* 2004). Similar systems have also been described for skeletal muscle (Rasmussen & Wolfe, 1999). ACC in the cytoplasm has rather low affinity for acetyl-CoA (Saddik *et al.* 1993; Poirier *et al.* 2002), and a decrease of this substrate due to changes in the kinetics of reactions in the Krebs cycle, as well as in acyl-carnitine transport and β-oxidation, naturally results in decreased malonyl-CoA production (Saddik *et al.* 1993; Schonekess & Lopaschuk, 1995; Reszko *et al.* 2004). Thus, the decrease of malonyl-CoA is *not the reason*, but *the consequence* of the increase in workload and fatty acid oxidation. Only if the workload decreases again can malonyl-CoA content increase and inhibit CPT I with the aim of avoiding overloading the mitochondria with fatty acid oxidation intermediates, which finally may uncouple oxidative phosphorylation and would thus have a deleterious effect (Neely & Morgan, 1974; Dyck & Lopaschuk, 2002; Taegtmeyer *et al.* 2005).

The conclusion from this analysis is that considering only one fragment (see grey area in Fig. 2) of the complex network of reactions may result in a misleading interpretation of the data. The explanation for why and how fatty acid oxidation is increased with increased work load can be found and clear answers given if all experimental data are analysed from the integrated system point of view in their historic perspective, as shown in the whole scheme in Fig. 2.

Molecular system analysis: integrated mechanisms of regulation of fatty acid and glucose oxidation

Figure 2 summarizes probably in the simplest possible way the network of reactions of main substrate supply for mitochondrial respiration in muscle cells and their multiple interactions and feedback mechanisms of regulation. This network has emerged from 70 years of research into muscle energy metabolism. The choice of the substrates for oxidation depends on their availability, and if glucose and FFAs are both present, FFAs strongly inhibits the transport of glucose across the plasma

would slow down oxidation of glucose and pyruvate (PYR) and increase glucose-6-phosphate (G6P), which would inhibit hexokinase (HK), and decrease glucose transport. The mitochondrial creatine kinase (miCK) catalyses the direct transphosphorylation of intramitochondrially produced ATP and cytosolic creatine (Cr) into ADP and phosphocreatine (PCr). ADP enters the matrix space to stimulate oxidative phosphorylation, while PCr is transferred via cytosolic Cr/PCr shuttle to be used by functional coupling of CK with ATPases (acto-myosin ATPase and ion pumps); If the workload increases, ATP production and respiration are increased due to feedback signalling via the creatine kinase (CK) system, leading to decreased mitochondrial content of acetyl-CoA, which is transferred into the cytoplasm with participation of carnitine acetyl carrier (CAC). Acetyl-CoA carboxylase (ACC) is responsible for converting acetyl-CoA to malonyl-CoA, a potent inhibitor of CPT I, with the aim to avoid overloading the mitochondria with fatty acid oxidation intermediates, when the workload is decreased. Inactivation of ACC occurs via phosphorylation catalysed by AMP-activated protein kinase (AMPK). Phosphorylation and inactivation of ACC leads to a decrease in the concentration of malonyl-CoA. A fall in malonyl-CoA levels disinhibits CPT I, resulting in increased fatty acid oxidation. Malonyl-CoA is also converted back into acetyl-CoA in the malonyl-CoA decarboxylase (MCD) reaction. Increase in the workload increases the rate of acetyl-CoA consumption and that automatically decreases the malonyl-CoA content. The ACC and MCD regulation occur under stress conditions when the AMP/ATP ratios are increased, but are unlikely to occour under normal work-load conditions of the heart. Thus, AMPK may be envisaged as a modulator, under situations of cellular stress, rather than as a master on–off switch of fatty acid oxidation.

membrane both in heart and in skeletal muscle (Neely & Morgan, 1974; Poirier *et al.* 2002; Roden, 2004). At relatively low workloads, mitochondrial acetyl-CoA and NADH produced by β-oxidation tend to inhibit the pyruvate dehydrogenase complex in the mitochondrial inner membrane, and citrate, the production of which is increased in the Krebs cycle, after transport across the inner mitochondrial membrane into the cytoplasm, inhibits PFK (Passoneau & Lowry, 1963; Neely & Morgan, 1974; Williamson *et al.* 1976; Williamson, 1979; Randle, 1998; Stanley *et al.* 2005). All these regulatory mechanisms explain the preference for FFAs for respiration in oxidative muscle cells. An important limitation of aerobic glycolysis is the necessity to maintain a rather low NADH/NAD^+ ratio in the cytoplasm needed for the high steady state flux through the glyceraldehyde phosphate dehydrogenase (GAPDH) reaction step, which is achieved by transfer of reducing equivalents into the mitochondrial matrix by the malate–aspartate shuttle (Williamson, 1979; Kobayashi & Neely, 1979). However, this shuttle becomes the rate limiting step at medium workloads (Kobayashi & Neely, 1979). The fatty acid pathway is free of this kind of limitation. During contraction, the regulation of all reactions of substrate supply starts with an increase in the workload, which under normal physiological conditions is usually governed by the Frank-Starling law (Saks *et al.* 2006). The red lines in Fig. 2 show the sequence of regulatory signals from cellular ATPases, like the acto-myosin ATPase for muscle contraction, to the mitochondrial matrix, which control fatty acid oxidation during workload changes. A workload-dependent increase in the rate of the actomyosin ATPase reaction results in a release of increasing amounts of ADP, and this metabolic signal is transmitted to the mitochondrial adenine nucleotide translocase (ANT) by phosphotransfer networks under conditions of apparent metabolic stability as described recently (Saks *et al.* 2006). Another component of the metabolic feedback signal to mitochondria is P_i, released from the splitting of PCr by the CK reaction (Wallimann *et al.* 1992) and subsequent hydrolysis of ATP by MgATPases. This P_i enters the mitochondria by a special phosphate carrier (Bose *et al.* 2003; Saks *et al.* 2006). These signals activate the ATP synthase and the utilization of the proton transmembrane electrochemical gradient ($\Delta\mu_H+$) for ATP synthesis in mitochondria (Hassinen & Hiltunen, 1975; Williamson, 1979; Hassinen, 1986; Nicholls & Ferguson, 2002). This process always leads to an increase in the electron transfer to oxygen via the respiratory chain and NADH and $FADH_2$ oxidation. Independently from the substrate used, an increased workload always decreases the ratio of NADH/NAD^+ in the mitochondrial matrix (Neely & Morgan, 1974; Hassinen & Hiltunen, 1975; Williamson *et al.* 1976; Williamson, 1979; Hassinen, 1986; Brandes & Bers, 1999). This decrease in the NADH/NAD^+ ratio on the other hand increases the rates of the dehydrogenase reactions in the Krebs cycle and gears the latter to the rate of electron transfer via the respiratory chain (Neely & Morgan, 1974; Williamson, 1979). It is the Krebs cycle, which is at the crossroads between the metabolic pathways of glucose and fatty acid oxidation, and its intermediates that play a very important role of feedback metabolic regulation of upstream pathways of substrate oxidation. This is not a simple, but a very complex system with the enzymes organized into multienzyme complexes and directly interacting by channelling of substrates and products (Velot *et al.* 1997), and the cycle intermediates are replenished by anaplerotic reactions (Nuutinen *et al.* 1981). However, there is a mitochondrial matrix pool of important intermediates of this cycle, which plays a role in feedback regulation of substrate oxidation (Williamson, 1979). One of the results of the activation of the reactions of the Krebs cycle is the strong decrease of the level of the acetyl-CoA in the mitochondrial matrix at high fluxes through this cycle, when the rate controlling steps are shifted towards the acyl-carnitine transporter and β-oxidation of fatty acids (Neely & Morgan, 1974). As described above, matrix acetyl-CoA can be rapidly exchanged with acetyl-CoA in cytoplasm by an equilibrium carrier system (CAC) with participation of carnitine (Lysiak *et al.* 1988; Saddik *et al.* 1993; DiLisa *et al.* 1995; Schonekess & Lopaschuk, 1995; Reszko *et al.* 2004). Thus, under this condition, a rapid fall in the acetyl-CoA content of the cytoplasm is the consequence (Neely *et al.* 1972; Oram *et al.* 1973), and it follows that the substrate for ACC in the cytoplasm simply disappears, and therefore malonyl-CoA decreases rapidly because of its decarboxylation by MCD back into acetyl-CoA, which is consumed in the Krebs cycle in the mitochondrial matrix (Schonekess & Lopaschuk, 1995; Reszko *et al.* 2004). This then releases the CPT I automatically from any inhibition, and FFA oxidation proceeds with a rate necessary for energy supply for contraction and ion pumps. A further interesting possibility, worthy of detailed study, is a probable compartmentation of AMPK at the sites of AMP production close to the acyl-CoA synthetase. This may be an additional, local mechanism of keeping the level of malonyl-CoA low and releasing CPT I from inhibition during fatty acid oxidation.

If now at some moments the workload is decreased again and less energy is needed, the reaction sequence described is reversed (see Fig. 1) and malonyl-CoA may increase to fulfil its role of negative feedback regulator of FFA oxidation by inhibiting CPT I to safeguard mitochondria from overload by acyl-CoA, which has detergent properties, and also may degrade to FFA and by uncoupling protein (UCP) may uncouple oxidative

phosphorylation (Neely & Morgan, 1974; Korge *et al.* 2003; Dyck & Lopaschuk, 2006).

Thus, in the heart, the changes in the malonyl-CoA content are most probably the consequences of alteration in FFA oxidation that is linearly related to changes in the workload, which regulates mitochondrial respiration by metabolic feedback signalling via phosphotransfer pathways. Thus, most probably, the reverse – that the FFA oxidation follows the changes in malonyl-CoA – may simply not be true, at least for the case of workload changes. The real role of malonyl-CoA is proposed to be clearly that of a negative feedback regulator of free fatty acid oxidation in the heart, as was correctly pointed out by Opie (1998). Thus, malonyl-CoA seems to be rather a metabolic feedback inhibitor, as many other intermediates in this complicated network of reactions are as well. For example, its role is similar to that of citrate in regulation of glycolysis by feedback inhibition of phosphofructokinase (PFK) (Passoneau & Lowry, 1963; Neely & Morgan, 1974). Usually, however, citrate is correctly not considered to be a key regulator of glycolysis.

This integrated network shown in Fig. 2 explains all of the main experimental observations on the regulation of respiration and substrate utilization in the heart. In short, the sequence of reactions in regulation of fatty acid and carbohydrate substrate oxidation in the heart may be formulated as follows: excitation–contraction coupling → activation of ATPases → feedback metabolic signalling via phosphotransfer networks → increase of respiration → increase of flux and changes of the kinetics in Krebs cycle → increase in fatty acid or glucose oxidation regulated by feedback mechanisms.

Malonyl-CoA seems to be rather clearly at the end of this sequence of events, and the AMPK signalling pathway apparently modifies only this last step of regulation under cellular stress conditions under which the [AMP]/[ATP] ratio increases significantly. Under these conditions the content of malonyl-CoA may indeed additionally be modified by AMPK, such that AMPK can be considered as a modulator, in particular under cellular stress situations, rather than as a master on–off switch of fatty acid oxidation. In heart with its particular metabolic stability, AMPK activation is only observed under conditions of ischaemia and/or reperfusion (Kudo *et al.* 1996; Dyck & Lopaschuk, 2002). In other tissues, the modulatory role of AMPK seems to be more pronounced even under physiological conditions (e.g. Dagher *et al.* 2001; Park *et al.* 2002; Assifi *et al.* 2005).

Skeletal muscles

For oxidative muscle cells, the integrated regulatory mechanisms described in Fig. 2 and the sequence of the reactions given above are probably valid as well, especially for some muscle types and under some conditions (endurance exercise training, etc.) (Rasmussen & Wolfe, 1999; Kiens, 2006). However, it should be emphasized that the mechanisms of regulation of skeletal muscle energy metabolism are in general very different from those for the heart and very different among distinct muscle fibre types (Kushmerick *et al.* 1992; Jorgensen *et al.* 2006). Interestingly, however, no changes in malonyl-CoA were found in skeletal muscles, as well, when fatty acid oxidation was increased during moderate or medium workloads, but the content of acetyl-CoA even increased slightly (Odland *et al.* 1998). In skeletal muscle cells, due to significant changes in the phosphocreatine content during exercises, and a following increase in [AMP]/[ATP] ratios, AMPK becomes quickly activated, and as a consequence, ACC is phosphorylated by AMPK and this decreases ACC activity and lowers the malonyl-CoA concentration, thus just decreasing the feedback regulation of fatty acid oxidation by malonyl-CoA (Winder & Hardie, 1996; Merrill *et al.* 1997; Park *et al.* 2002). However, in skeletal muscles, the analysis of biopsy samples is always complicated because of the differences in fibre type composition of human muscle. Most interestingly, very recent studies show that in skeletal muscle, the malonyl-CoA sensitive step of import of fatty acids into mitochondria may be bypassed by another mitochondrial long-chain fatty acid transport protein (Holloway *et al.* 2006). Thus, this very specific metabolic question of the regulation of fatty acid oxidation by malonyl-CoA in skeletal muscle should be studied in more detail again, accounting for the integrated regulatory network described in Fig. 2. If in skeletal muscle AMPK were indeed the master switch for regulation of fatty acid oxidation, an activator of this enzyme could be envisaged as a pharmacological strategy against obesity and metabolic syndrome. However, knowing that AMPK activation is needed for progression of certain cancers via induction of vascular endothelial growth factor (VEGF) to make tumours tolerant to ischaemia (Lee *et al.* 2006) may still preclude such a strategy, for global activation of AMPK for inducing the 'burning of fat' may favour the survival of a pre-existing cancer.

Finally, to understand the complex network of cellular regulatory systems, it seems to be essential to include all layers of regulation in a systems biology approach, including, for example, classical metabolic regulation together with more recent advances in cellular signalling cascades. It is clear, however, that further quantitative analyses of all these metabolic interactions in the network of substrate supply and utilization, both by experiments and mathematical modelling are challenging and urgent tasks for molecular system bioenergetics in the future.

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